

# Monarch<sup>®</sup> RNA Purification

HIGH PERFORMANCE, SUSTAINABILITY & VALUE



**RNA**

# Make the better choice and migrate to Monarch

Monarch® Nucleic Acid Purification Kits are the perfect complement to many molecular biology workflows, offering exceptional value for a range of budgets. Recover pure, intact DNA and RNA in minutes with our fast, user-friendly protocols and optimized buffer systems, and focus your time on the experiments that will drive your research forward. The Monarch nucleic acid purification portfolio can serve your needs, whether you are isolating nucleic acids from biological samples, cleaning up DNA and RNA from enzymatic reactions, extracting DNA fragments from gels, or purifying plasmids.

Monarch kits are all designed with sustainability in mind; kits and spin column components are made with significantly less plastic than leading suppliers, and are packaged with responsibly-sourced, recyclable packaging.

## Available kits:

### Monarch Spin RNA Isolation Kit (Mini) (NEB [#T2110](#))

- Extract total RNA from a variety of sample types with this comprehensive kit that includes Proteinase K and stabilization reagent
- Tested with cells, blood, tissues, bacteria, yeast, plant, insects and more

### Monarch Spin Kits for RNA Cleanup (NEB [#T2030](#), [#T2040](#), [#T2050](#))

- Purify and concentrate RNA after enzymatic reactions and RNA synthesis
- Choose between three binding capacities (10 µg, 50 µg, and 500 µg)

### Monarch Mag Viral DNA/RNA Extraction Kit (NEB [#T4010](#))

- Hands-free extraction of viral DNA and/or RNA using a magnetic bead-based protocol
- Compatible with automated high-throughput workflows on a variety of platforms

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Visit [NEBMonarch.com](https://www.neb.com/monarch) to learn more and request a sample.

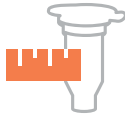
# Monarch kits are designed for sustainability and value



## Reduced lab waste



**Significantly less plastic as compared to leading supplier**  
Monarch kits still deliver high yields, purity and performance



**Thinner-walled columns**  
Reduction in total plastic without affecting performance



**Buffer bottles**  
Carefully designed to minimize plastic usage



## Flexible purchasing options



**Buffers and columns sold separately**  
Purchase only what you need and avoid wasted materials



**Same performance, design and formulations**  
Standalone products are the same components that are included in complete kits



**MONARCH®**  
Sustainability



## No excessive packaging



**Sturdy, reusable boxes at just the right size**  
Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere



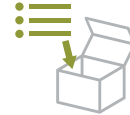
**Concise protocol cards replace printed manuals**  
Manuals are available online



## Sustainable & recyclable packaging



**Sourced for recyclability**  
All components are purposefully sourced for recyclability



**Instructions for recycling kit components**  
Can be found online

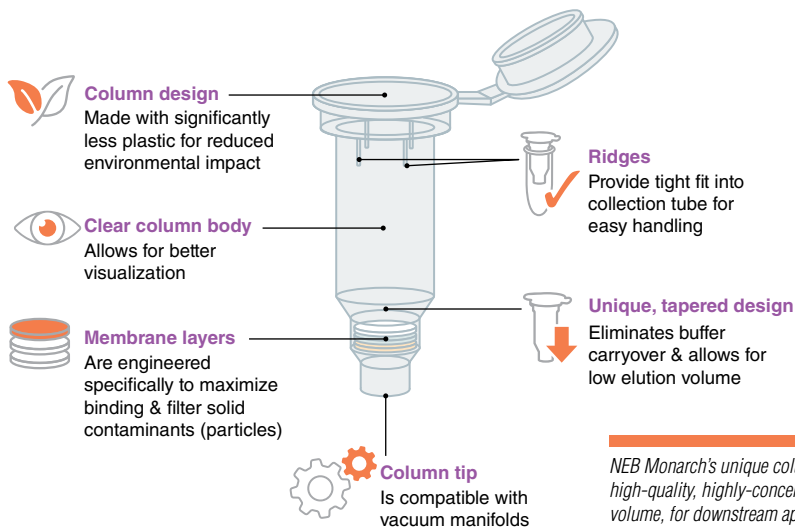


**Recycled paper**  
Used to make the kit boxes, inserts and paper materials



**Eco-friendly printing**  
Printing of boxes and packaging powered by green sustainable sources such as wind

## Features of the Monarch Spin Column for Nucleic Acid Purification



*NEB Monarch's unique column design and membrane assembly allows high-quality, highly-concentrated nucleic acid purification with low elution volume, for downstream applications. The column is designed and made with significantly less plastic for a reduced environmental impact.*

# Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110)

The Monarch Spin RNA Isolation Kit (Mini) is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from various biological samples, including cultured cells, mammalian tissues, microbes, plants, insects and blood. With just this single kit, users can purify up to 100 µg of high-quality total RNA from multiple sample types, from standard as well as tough-to-lyse samples, with included enzymes, reagents, and specialized gDNA removal columns. The kit uniquely enables binding capacities like RNA purification ‘mini’ kits, combined with the low elution volumes of ‘micro’ kits. Cleanup of enzymatic reactions and purification of RNA from TRIzol®-extracted samples is also possible using this kit. Purified RNA has metrics with A<sub>260/280</sub> and A<sub>260/230</sub> ratios typically > 2.0, high RNA integrity scores, and minimal residual gDNA. This kit captures RNA ranging in size from full-length rRNAs down to intact miRNAs. Purified RNA is suitable for downstream applications such as RT-qPCR, cDNA synthesis, RNA-seq, and RNA hybridization-based technologies.

## ADVANTAGES

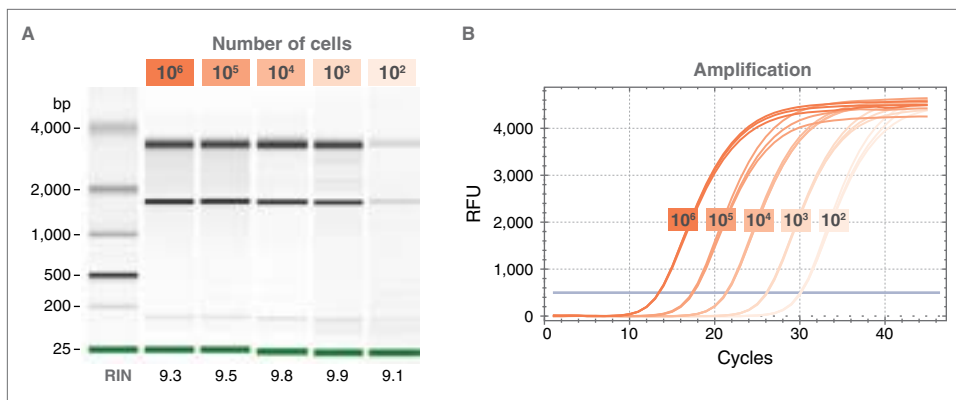
- Use with a wide variety of sample types, including cells, fibrous or lipid-rich tissues, bacteria, yeast, plants, insects and more
- Purify RNA of all sizes, including small RNAs < 200 nucleotides
- Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent, Monarch StabiLyse DNA/RNA Buffer (NEB #T2111)

## SPECIFICATIONS

- **Binding Capacity:** 100 µg RNA
- **RNA Size:** ≥ 20 nt
- **Purity:** A<sub>260/280</sub> and A<sub>260/230</sub> usually ≥ 1.8
- **Input Amount:** up to 10<sup>6</sup> cells or 10 mg tissue\*
- **Elution Volume:** 30–100 µl
- **Yield:** varies depending on sample type
- **Compatible downstream applications:** cDNA synthesis, RT-qPCR, RNA-Seq, small RNA library prep, Northern blotting, hybridization-based workflows

\*See page 6 for more details and other sample types

Monarch Spin RNA Isolation Kit (Mini) enables extraction of high-quality RNA even from low input samples

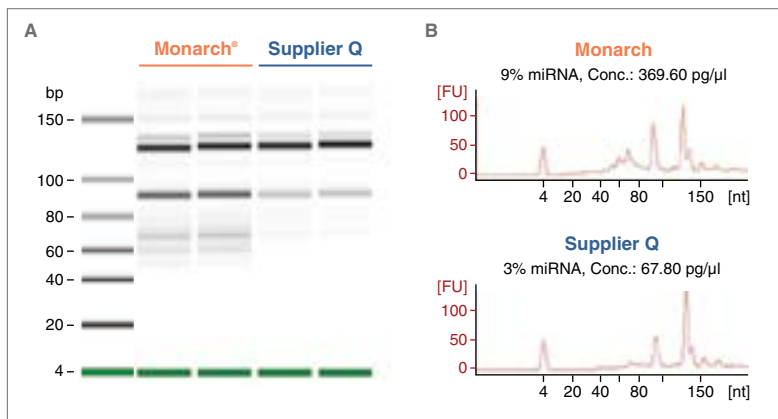


RNA was extracted using the Monarch Spin RNA Isolation Kit (Mini) from varying amounts of HEK293 cells over 5 orders of magnitude starting from 1 million cells to 100. A. To assess RNA integrity, the extracted RNA was resolved using the Agilent® Pico Bioanalyzer chip on a Bioanalyzer 2100 with sample loading adjusted to the manufacturer's recommendation of assay input range. B. To demonstrate successful downstream application, the RNA was subjected to RT-qPCR using Luna® Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) in a 20 µl reaction targeting GAPDH mRNA. Expected amplification curves were observed showing a quantitative trend corresponding to the varying input amounts.

“ I compared the yield of RNA extracted and the purity of the RNA...when I compared to my usual RNeasy [Plant Mini] kit to the Monarch sample, I obtained 5 times more RNA from my sample, as well as higher purity of my sample with... Monarch.”

– Researcher studying oat protoplast cells, Iowa State University

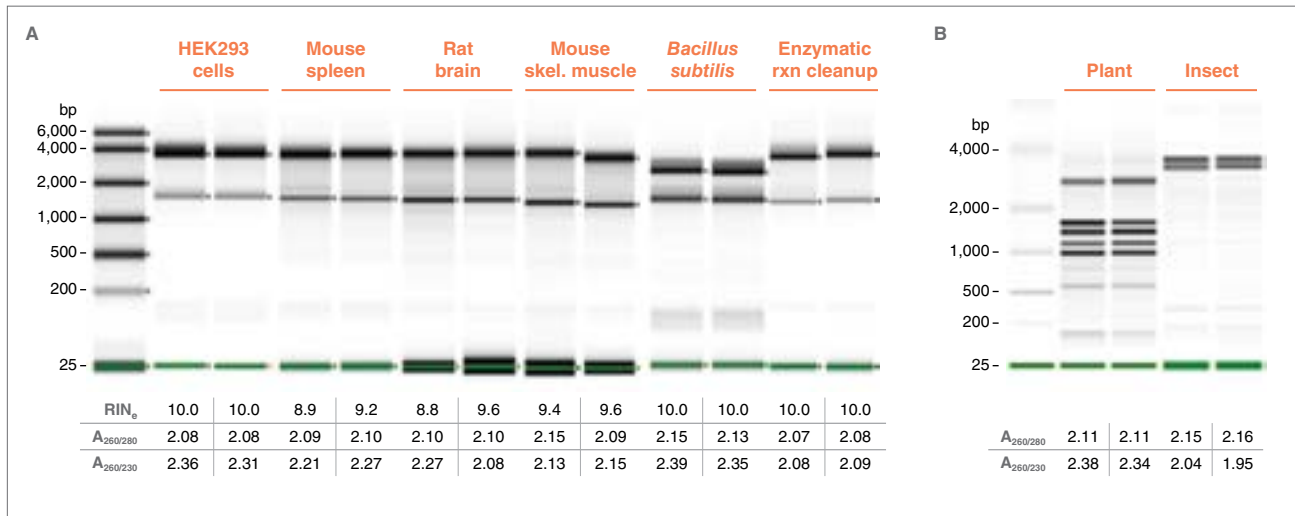
Monarch Spin RNA Isolation Kit (Mini) successfully purifies small RNAs below 200 nucleotides, enabling a more faithful representation of the total RNA pool compared to a leading supplier



RNA was extracted from 10 mg mouse brain tissues using Monarch Spin RNA Isolation Kit (Mini) and a comparable kit from Supplier Q. To visualize the isolation of small RNA species, the extracted RNA was resolved using the Agilent Small RNA chip on a Bioanalyzer 2100 according to manufacturer's instructions. Gel traces of RNA isolated from the two kits are shown (A), with representative electropherograms showing the small RNA size profiles (B).

# Monarch-purified RNA is High-quality and Compatible with a Wide Variety of Downstream Applications

Monarch Spin RNA Isolation Kit (Mini) is a versatile solution for high-quality RNA extraction from a wide variety of sample types



RNA was extracted in duplicate using the Monarch Spin RNA Isolation Kit (Mini) from various samples representing a range of biological properties in the starting material, including fibrous, fatty and nuclease-rich mammalian tissues, tough-to-lyse samples such as Gram-positive bacteria, plants and insects, as well as RNA cleanup reactions. RNA quality was assessed using A260/A280 and A260/A230 ratios measured using a microvolume spectrophotometer (Lunatic<sup>®</sup>, Unchained Labs<sup>®</sup>). RNA integrity was measured using Agilent<sup>®</sup> automated electrophoresis systems according to sample types. For samples with known typical RNA profiles, Agilent RNA ScreenTape<sup>®</sup> was used on the TapeStation<sup>®</sup> 4200 (A). For samples with known atypical RNA profiles such as plant green tissue with plastidal content, and insects with ribosomal gene breaks, Agilent Bioanalyzer<sup>®</sup> 2100 used with NanoChip (B).

## TIPS FOR SUCCESSFUL RNA EXTRACTIONS

- 1. Prevent RNase Activity:** Nucleases in your sample will degrade RNA, so inhibiting their activity is essential. Process samples quickly after harvest, use preservation reagents, and always ensure you are working in nuclease-free working environments.
- 2. Inactivate RNases after harvesting your sample:** Nucleases in your sample will lead to degradation, so inactivating them is essential. Process samples quickly, or use preservation reagents, and always ensure nuclease-free working environments.
- 3. Do not exceed recommended input amounts:** Buffer volumes are optimized for the recommended input amounts. Exceeding these can result in inefficient lysis and can also clog the column. See page 6.
- 4. Ensure samples are properly homogenized/disrupted:** Samples should be disrupted and homogenized completely to release all RNA.
- 5. For sensitive applications, ensure proper gDNA removal:** gDNA is removed by the gDNA removal column (Monarch Spin Column S2C) and subsequent on-column DNase I treatment. Off-column DNase I treatment can also be employed.

## DOING qPCR OR RT-qPCR?

Learn more about Luna and request a sample at [LUNAqPCR.com](http://LUNAqPCR.com)

## Choosing Sample Input Types for RNA Purification and Average Yields Expected

Below, we have provided some empirical yield, purity, and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110). It is important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

SAMPLE TYPE		RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	OBSERVED RIN or RINe	MAXIMUM INPUT AMOUNT
<b>CULTURED CELLS</b>					
HEK 293		1 x 10 <sup>6</sup> cells	12–14	9–10	15 x 10 <sup>6</sup> cells
NIH3T3		1 x 10 <sup>6</sup> cells	8–12	9–10	1 x 10 <sup>7</sup> cells
<b>TISSUE</b>					
Rat liver (frozen stabilized)		10 mg	40–60	8–9	20 mg
Rat spleen (frozen stabilized)		10 mg	40–50	9	20 mg
Rat kidney (frozen stabilized)		10 mg	7–10	9	50 mg
Rat brain (frozen stabilized)		10 mg	5–8	8–9	50 mg
Rat muscle (frozen stabilized)		10 mg	2–3	8–9	50 mg
Mouse heart (frozen stabilized)		10 mg	5–6	8–9	50 mg
<b>BLOOD OR PLASMA</b>					
Human	Fresh	200 µl	0.5–1.0	7–8	200 µl
	Frozen	200 µl	0.5–1.0	7–8	200 µl
Rat	Frozen	200 µl	5–6	9	200 µl
<b>BLOOD CELLS</b>					
PBMC (isolated from 5 ml whole blood)		5 ml	1–3	7	5 x 10 <sup>6</sup> cells
<b>YEAST</b>					
<i>S. cerevisiae</i>	Frozen with bead homogenizer	1 x 10 <sup>7</sup> cells	20–40	9–10	5 x 10 <sup>7</sup> cells
	Frozen with Zymolyase®	1 x 10 <sup>7</sup> cells	20–40	9	5 x 10 <sup>7</sup> cells
<b>BACTERIA</b>					
<i>E. coli</i>	Frozen pellet with bead homogenizer	1 x 10 <sup>9</sup> cells	10–15	10	1 x 10 <sup>9</sup> cells
	Frozen pellet with lysozyme	1 x 10 <sup>9</sup> cells	40–60	10	1 x 10 <sup>9</sup> cells
<i>B. subtilis</i>	Frozen pellet with bead homogenizer	1 x 10 <sup>9</sup> cells	15–20	9	1 x 10 <sup>9</sup> cells
	Frozen pellet with lysozyme	1 x 10 <sup>9</sup> cells	20–30	9–10	1 x 10 <sup>9</sup> cells
<b>PLANT</b>					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Onion leaf (fresh with bead homogenizer)		50 mg	4–6	8*	50 mg
Root		50 mg	8–10	8*	50 mg
<b>INSECTS</b>					
Mosquito (preserved in ethanol dry ice bath)		10 mg	20–30	9*	20 mg
House fly (preserved in ethanol dry ice bath)		10 mg	10–20	9*	10 mg

RNA yields and RINs observed during evaluation are reported here. RNA yield and quality are influenced by several factors including sample growth stage and RNA content, storage condition, sample handling during processing as well as the chosen method of sample lysis and homogenization. It is crucial to consider these factors and use best practices to maximize RNA yield and quality. Please refer to Important Notes in the Product Manual before starting for recommendations on Working with RNA and Considerations for Sample Lysis and Homogenization.

\*These samples contain RNA with an atypical ribosomal profile that is not applicable for standard RIN measurement. Users are encouraged to set their own threshold or assess RNA integrity by visually inspecting the bands generated by the automated gel electrophoresis platform.



# Monarch Spin Kits for RNA Cleanup

(NEB [#T2030](#), [#T2040](#), [#T2050](#))

The Monarch Spin RNA Cleanup Kits provide a fast and simple silica column-based solution for cleanup and concentration of RNA after enzymatic reactions (including *in vitro* transcription (IVT), DNase I and Proteinase K treatment, capping, tailing and labeling) as well as after RNA isolation (e.g., TRIzol extraction). These kits can also be used to extract RNA from cells, saliva and buccal/nasopharyngeal swabs. Kits are available in three different binding capacities: 10 µg, 50 µg and 500 µg, each containing unique columns designed to prevent buffer retention and ensure no carryover of contaminants.

## Monarch Kit Specifications:

MONARCH SPIN RNA CLEANUP KIT	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
<b>Binding Capacity</b>	10 µg	50 µg	500 µg
<b>RNA Size Range</b>	≥ 25 nt (≥ 15 nt with modified protocol)		
<b>Typical Recovery</b>	70–100%		
<b>Elution Volume</b>	6–20 µl	20–50 µl	50–100 µl
<b>Purity</b>	$A_{260/280} > 1.8$ and $A_{260/230} > 1.8$		
<b>Protocol Time</b>	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time
<b>Common Downstream Applications</b>	RT-PCR, RNA library prep for NGS, small RNA library prep for NGS, RNA labeling	RT-PCR, RNA library prep for NGS, formation of RNP complexes for genome editing, microinjection, RNA labeling, transfection	RT-PCR, RNA library prep for NGS, RNA labeling, RNAi, microinjection, transfection

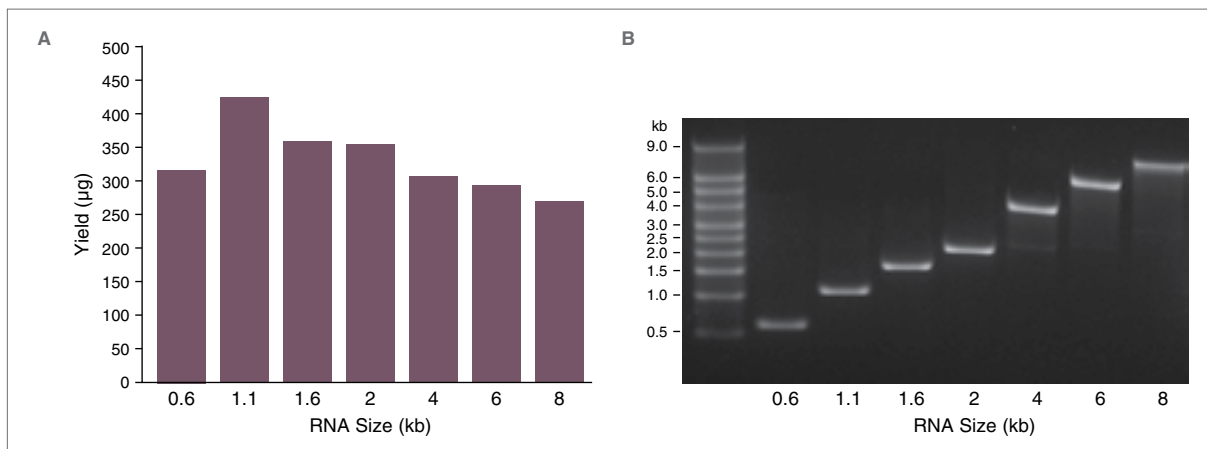
## ADVANTAGES

- Isolate highly pure RNA ( $A_{260/280}$  and  $A_{260/230} \geq 1.8$ ) in minutes
- Clean up RNA with simple protocol utilizing a single wash buffer
- Elute in as little as 6 µl (NEB #T2030) or 20 µl (NEB #T2040)
- Bind up to 500 µg of RNA (NEB #T2050)
- Adjust cutoff size down to 15 nt with a slight protocol modification
- Can be used for RNA extraction from some samples

## APPLICATIONS

- Cleanup & concentration after enzymatic reactions (e.g., DNase I and Proteinase K treatment)
- Cleanup after RNA synthesis (IVT and sgRNA synthesis)
- Cleanup & concentration of previously-purified RNA (e.g., after TRIzol extraction)
- RNA extraction from cells, saliva and swabs (buccal/NP)
- RNA Gel Extraction

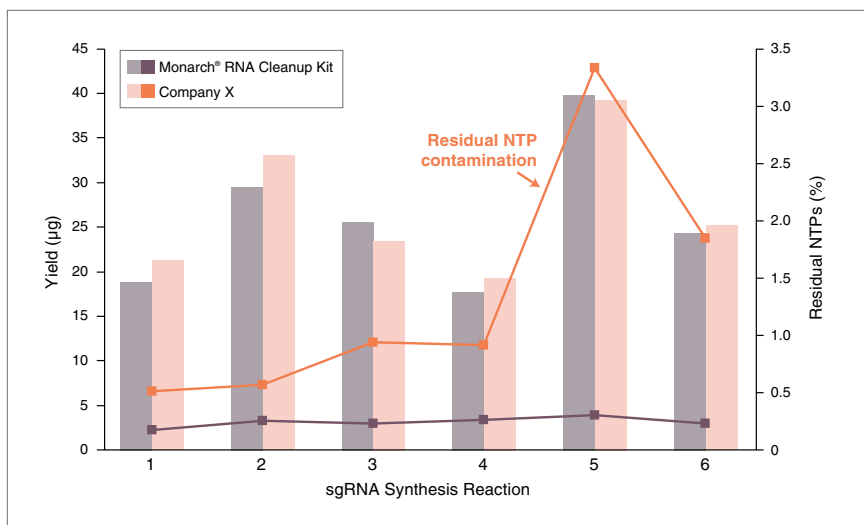
The Monarch Spin RNA Cleanup Kit (500 µg) is suitable for cleaning up large quantities (> 250 µg) of RNA from *in vitro* transcription reactions



A. RNA transcripts of varying sizes (0.6–8 kb) were synthesized using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (NEB #E2050). 40 µl of each *in vitro* transcription (IVT) reaction was cleaned up using the Monarch Spin RNA Cleanup Kit (500 µg) (NEB #T2050). RNA yields were calculated from the resulting  $A_{260}$  measured using a Nanodrop spectrophotometer and ranged from 268–425 µg of RNA per IVT reaction.

B. RNA integrity (200 ng/lane) was assessed on a 1% agarose-TBE gel stained with SYBR® Gold.

The Monarch Spin RNA Cleanup Kit (50 µg) produces sgRNA yields consistent with other competitor RNA cleanup kits and with lower residual NTP contamination

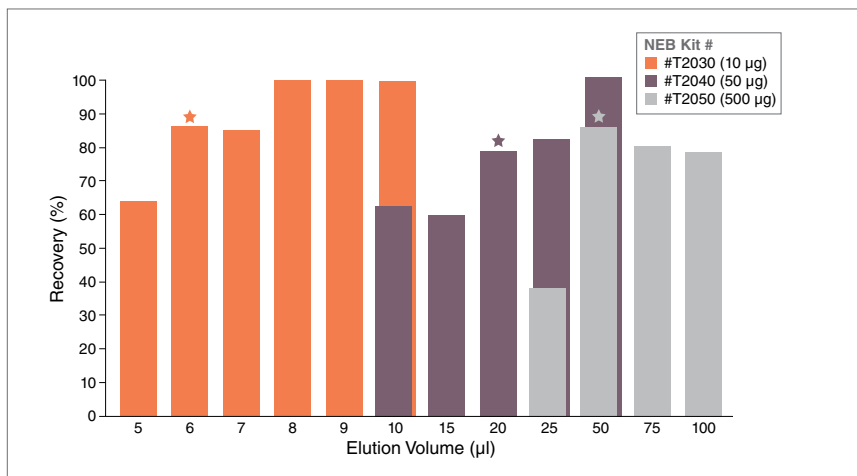


Six different sgRNA synthesis reactions from the EnGen® sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) were cleaned up using either the Monarch Spin RNA Cleanup Kit (50 µg, NEB #T2040) or a competitor kit (according to manufacturer's recommendations) and eluted in 50 µl nuclease-free water. sgRNA yield was calculated from the resulting  $A_{260}$  measured using a Trinean DropSense 16. The Monarch Spin RNA Cleanup Kit produced sgRNA yields consistent with other commercially available RNA cleanup kits.

Following cleanup, residual nucleotides (NTPs) were measured by LC-MS and are reported as percent area NTPs ( $rATP+rCTP+rGTP+rUTP$ )/percent area sgRNA. The NEB Monarch Spin RNA Cleanup Kit consistently outperforms other commercially available RNA cleanup kits in the removal of residual NTPs from sgRNA synthesis reactions.

Learn more about NEB's reagents for synthesis of high-quality RNA – from template generation and transcription, capping, tailing, and cleanup after synthesis. Visit [neb.com/IVT](http://neb.com/IVT).

Recovery of RNA from Monarch Spin RNA Cleanup Kits with varying elution volumes



10, 50 or 500 µg of RNA (16S and 23S Ribosomal Standard from *E. coli*, Sigma) was purified using a Monarch Spin RNA Cleanup Kit (10 µg, #T2030) (50 µg, #T2040) (500 µg, #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting  $A_{260}$  as measured using a Trinean DropSense 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch Spin RNA Cleanup Kit (10 µg, #T2030), 20 µl from the Monarch Spin RNA Cleanup Kit (50 µg, #T2040), and 50 µl from the Monarch Spin RNA Cleanup Kit (500 µg, #T2050).

“ We recently switched to NEB for our spin column kits, and I just wanted to say that I am so impressed with the thoughtfulness of the packaging—how the protocol is stored, the tiny bags that the columns are in, and the quality of the box itself, which we will keep to store items after we finish the kit. We also like that you can customize the elution volume. Cheers to your team for doing your part and designing a great product!

– Jaclyn, George Washington University

Learn more at [NEB.com/MonarchRNACleanup](http://NEB.com/MonarchRNACleanup).



# Troubleshooting Guide for Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110)

Our troubleshooting guide below outlines some of the most common pain points scientists encounter during RNA purification.

PROBLEM	COMMON CAUSE	SUGGESTIONS/SOLUTIONS
<b>Column clogging</b>	Sample input higher than recommended	<ul style="list-style-type: none"> <li>Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Refer to recommendations under Choosing Sample Input Types.</li> </ul>
	Insufficient Lysis	<ul style="list-style-type: none"> <li>Increase time of digestion or homogenization.</li> <li>Centrifuge sample to pellet debris and use only supernatant for next steps.</li> <li>Use larger volume of buffer for lysis and homogenization.</li> </ul>
<b>Low RNA yield</b>	Insufficient Lysis	<ul style="list-style-type: none"> <li>Increase time of digestion or homogenization.</li> <li>Centrifuge sample to pellet debris and use only supernatant for next steps.</li> <li>Use larger volume of buffer for lysis and homogenization.</li> </ul>
	Sample is degraded	<ul style="list-style-type: none"> <li>Use RNA preservation reagents to maintain RNA integrity during storage.</li> <li>Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible.</li> <li>To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE.</li> <li>See Product Manual under Important Notes Before Starting.</li> </ul>
	Sample input higher than recommended	<ul style="list-style-type: none"> <li>Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Overloading leads to inefficient purification, insufficient elution and reduced yield. See Choosing Input Amounts.</li> </ul>
<b>Low RNA quality</b>	Sample is degraded	<ul style="list-style-type: none"> <li>Use RNA preservation reagents to maintain RNA integrity during storage.</li> <li>Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible.</li> <li>To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE.</li> </ul>
	Salt/Ethanol carryover	<ul style="list-style-type: none"> <li>Low <math>A_{260/230}</math> values indicate residual guanidine salts have been carried over during elution. Ensure wash steps are carried out prior to eluting sample. Do not skip any washes with Buffer BX and Buffer WZ.</li> <li>Use care to ensure the column tip does not contact the flow-through. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a soft paper towel prior to reattachment to the column to remove any residual wash buffer.</li> <li>Add additional wash step and/or extend spin time for final wash.</li> </ul>
	Residual protein carryover	<ul style="list-style-type: none"> <li>Low <math>A_{260/280}</math> values indicate residual protein in the purified sample. Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA purification column. Do not skip any washes with Buffer BX and Buffer WZ.</li> </ul>
<b>DNA contamination</b>	DNA carryover	<ul style="list-style-type: none"> <li>Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample.</li> <li>Perform in-tube/off-column DNase I treatment to remove gDNA. See Appendix in Product Manual.</li> </ul>
	Sample input higher than recommended	<ul style="list-style-type: none"> <li>Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded.</li> </ul>
<b>Low performance of RNA in downstream steps</b>	Salt and/or ethanol carryover has occurred	<ul style="list-style-type: none"> <li>Use care to ensure the column tip does not contact the flow-through after the final wash. If unsure, please repeat centrifugation.</li> <li>Be sure to spin the column for 2 minutes following the final wash with Monarch Buffer WZ.</li> <li>When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.</li> <li>Add additional wash step and/or extend spin time for final wash.</li> </ul>
<b>Unusual Spectrophotometric readings</b>	RNA concentration is too low for spectrophotometric analysis	<ul style="list-style-type: none"> <li>For more concentrated RNA, elute with 10 <math>\mu</math>l of nuclease-free water.</li> <li>Increase amount of starting material (within kit specifications). See Choosing Input Amounts or Product Manual.</li> </ul>
	Silica fines in eluate	<ul style="list-style-type: none"> <li>Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the <math>A_{260/230}</math> is unaffected by possible elution of silica particles.</li> </ul>

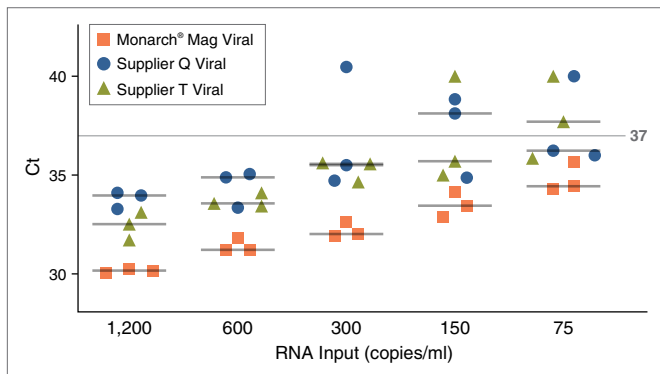
# Troubleshooting Guide for Monarch Spin RNA Cleanup Kits (NEB [#T2030](#), [#T2040](#), [#T2050](#))

PROBLEM	CAUSE	SOLUTION
<b>Low RNA yield</b>	Reagents added incorrectly	<ul style="list-style-type: none"> <li>• Check protocol to ensure correct buffer reconstitution, order of addition for buffers and ethanol, and proper handling of column flow-through and eluents</li> </ul>
	Insufficient mixing of reagents	<ul style="list-style-type: none"> <li>• Ensure the ethanol is thoroughly mixed with mixture of RNA sample and RNA binding buffer before applying sample to the purification column</li> </ul>
	Incomplete elution during prep	<ul style="list-style-type: none"> <li>• Ensure the nuclease-free water used for elution is delivered directly to the center of the column so that the matrix is completely covered and elution is efficient. Larger elution volumes and longer incubation times can increase yield of RNA off the column at the cost of dilution of the sample and increased processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient. Additionally, multiple rounds of elution can be employed to increase the amount of RNA eluted, at the expense of dilution of the sample. The first elution can be used to elute a second time to maximize recovery and minimize sample dilution.</li> </ul>
	Complex secondary structure affects binding and elution of smaller RNAs (< 45 nt)	<ul style="list-style-type: none"> <li>• Diluting your sample with 2 volumes of ethanol instead of one volume in Step 2.</li> </ul>
	Poor extraction from Agarose gel	<ul style="list-style-type: none"> <li>• Be sure to incubate the sample between 37–55°C after addition of both the RNA cleanup binding buffer and the ethanol. Additionally, incubate the column with nuclease-free water at 65°C for 5 minutes prior to spinning to elute the RNA.</li> </ul>
<b>Purified RNA is degraded</b>	RNase contamination	<ul style="list-style-type: none"> <li>• Wear gloves and use disposable RNase-free tips (not provided) and collection tubes during the procedure</li> <li>• Keep all kit components tightly sealed when not in use</li> </ul>
	Improper storage of RNA	<ul style="list-style-type: none"> <li>• Use RNA immediately in downstream applications or store at -70°C</li> </ul>
<b>Low OD ratios</b>	Residual guanidine salts have been carried over during elution	<ul style="list-style-type: none"> <li>• Ensure wash steps are carried out prior to eluting sample</li> <li>• Use care to ensure the tip of the column does not contact the flow-through. If unsure, please repeat centrifugation</li> <li>• When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer</li> </ul>
<b>Low performance of RNA in downstream steps</b>	Salt and/or ethanol carry-over	<ul style="list-style-type: none"> <li>• Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over in the eluted RNA</li> </ul>
<b>DNA contamination</b>	DNA present in sample	<ul style="list-style-type: none"> <li>• Incubate RNA sample with DNase I (NEB #M0303) and clean up RNA using the Monarch Spin RNA Cleanup Protocol</li> </ul>

# Monarch Mag Viral DNA/RNA Extraction Kit (NEB #T4010)

The Monarch Mag Viral DNA/RNA Extraction Kit provides a rapid and reliable magnetic bead-based process for extracting viral nucleic acids from saliva and respiratory swab samples. The kit combines the efficiency of silica-based nucleic acid purification with the ease of use of magnetic beads. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Kit sizes align to 96-well formats (100 preps, 600 preps, and 1800 preps), and the protocol is compatible with high throughput automation on a variety of platforms, including the KingFisher Flex magnetic particle processor and Agilent Bravo and MGISP liquid handler platforms.

Performance comparison of Monarch Mag Viral DNA/RNA Extraction Kit with other suppliers demonstrates high reproducibility and sensitivity



Mock samples representing decreasing viral loads were prepared using Heat-inactivated SARS-CoV-2 (ATCC) in VTM (Hardy Diagnostics<sup>®</sup>). Extraction was performed using Monarch Mag Viral DNA/RNA Extraction Kit and similar kits from two other suppliers. RT-qPCR was performed using NEB #E3019 and BioRad CFX96 Touch Real-Time PCR Detection System. Monarch Mag Viral DNA/RNA Extraction Kit showed consistently low Ct's and reproducible data, even at low viral loads, compared to the competitor kits tested.

## ADVANTAGES

- Designed for hands-free extraction of viral DNA and/or RNA
- Utilizes magnetic bead-based methods. Compatible with manual and automated high-throughput workflows on a variety of instrument platforms, such as KingFisher<sup>®</sup> Flex, Agilent<sup>®</sup> Bravo<sup>®</sup>, MGISP<sup>®</sup> liquid handlers, and more
- Tested for saliva and respiratory swab sample types. Compatible with wastewater samples, after enrichment steps (not supplied)
- Suitable for qPCR/RT-qPCR, ddPCR, library prep for sequencing/NGS and other downstream applications
- Includes carrier RNA for sensitive detection in RNA-based amplification workflows

## Products for RNA Purification

PRODUCT	NEB #	SIZE
Monarch Spin RNA Isolation Kit (Mini)	<a href="#">T2110S</a>	50 preps
Monarch Spin RNA Cleanup Kit (10 µg)	<a href="#">T2030S/L</a>	10/100 preps
Monarch Spin RNA Cleanup Kit (50 µg)	<a href="#">T2040S/L</a>	10/100 preps
Monarch Spin RNA Cleanup Kit (500 µg)	<a href="#">T2050S/L</a>	10/100 preps
<b>COLUMNS AVAILABLE SEPARATELY</b>		
Monarch Spin Columns S2C	<a href="#">T3017L</a>	100 columns and tubes
Monarch Spin Columns S2A	<a href="#">T2047L</a>	100 columns and tubes
Monarch Spin Collection Tubes	<a href="#">T2118L</a>	100 tubes
Monarch Spin Columns S1A	<a href="#">T2037L</a>	100 columns and tubes
Monarch Spin Columns S2B	<a href="#">T2057L</a>	100 columns and tubes
<b>BUFFERS &amp; REAGENTS AVAILABLE SEPARATELY</b>		
Monarch StabiLyse™ DNA/RNA Buffer	<a href="#">T2111L</a>	145 ml
Monarch Buffer BX	<a href="#">T2041L</a>	80 ml
Monarch Buffer WX	<a href="#">T2042L</a>	40 ml
Monarch Buffer WZ	<a href="#">T1115L</a>	26 ml
Nuclease-free Water	<a href="#">B1500S/L</a>	25 ml/100 ml



**Time for change – try a Monarch sample**  
 Try a sample of our Monarch Nucleic Acid Purification Kits by visiting [NEBMonarch.com](https://www.neb.com)

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